Conférence annuelle du GdR Phycotox

IUEM Plouzané (31 mai - 1er juin 2022)

Book of abstracts

Présentations orales

Cytotoxic and genotoxic properties of C17-Sphinganine analog mycotoxin evaluated on human hepatic HepaRg and lymphoblastoid TK6 cell lines

Zeineb Marzougui **1, 2,** Lehegarat Ludovic **2** , Kevin Hogeveen **2,** Sylvie Huet **2,**, Riadh Kharrat **1**, Valérie Fessard **2, ***, Riadh Marrouchi **1, ***

1 : Venom and therapeutic biomolecules Laboratory, Institut Pasteur de Tunis, Tunis El Manar University, 13, Place Pasteur, B.P 74, 1002 Belvédère, Tunis - Tunisie

2 : Toxicology of contaminants Unit, French Agency for Food, Environmental and Occupational Health Safety, Agence nationale de sécurité sanitaire de l\'alimentation, de l\'environnement et du travail, 10 bis Rue Claude Bourgelat, 35133 Javené - France

* : Auteur correspondant

Severe toxicity was observed in mussels from Bizerte Lagoon (Northern Tunisia) using routine mouse bioassay for detecting diarrheic and paralytic toxins although not associated to classical phytoplankton blooming. C17-sphinganine analog mycotoxin (C17-SAMT), with a molecular mass of 287.289 Da, was identified as the toxic compound responsible for this atypical toxicity, characterized by rapid death of mouse injected intraperitoneally with contaminated mussel's extracts. Electrophysiological studies on the mouse neuromuscular system demonstrated that C17-SAMT exerts its effects by blocking skeletal muscle contraction, which can explain some of the symptoms observed during acute toxicity assays. The lethal doses (LD50) of C17-SAMT by i.c.v., i.p., and per os routes are, respectively, 150 µg/kg, 750 µg/kg, 900 µg/kg. Concerning in vitro genotoxicity, C17-SAMT induced micronuclei formation in TK6 cells after 3 and 24h of exposure at 250 µg/ml and 500 µg/ml. However, no increase of DNA damage was detected in TK6 cells with the alkaline and modified (FpG) comet assay. Furthermore, an increase in phospho-H3 but not in γ-H2Ax nuclear markers was observed in proliferative HepaRG cells treated by C17-SAMT for 24h. When investigating the toxic effects of C17-SAMT on differentiated HepaRG cells, we reported an increase in mitochondrial transmembrane potential by TMRE immunostaining after 1 to 24h of exposure at 125, 250 and 500 µg/ml. C17-SAMT at 500 µg/ml also increased superoxide dismutase in HepaRG cells following 24h treatment. Moreover, a decrease of NFκB intensity in both cytoplasm and nucleus, suggesting a degradation process, was observed. In conclusion, C17-SAMT is genotoxic in vitro. Although some clues have been provided in this study, further experiments are needed to elucidate the mechanisms involved in C17-SAMT toxicity. The genotoxic effect will be also assessed by in vivo studies.

Mots-Clés : Marine toxins ; mycotoxins ; genotoxicity ; HepaRg ; TK6

Multi-omics characterization of portimine and pinnatoxin-G effects and metabolization on the human liver HepaRG cell line

Léonie Dec **1,** Valérie Fessard **1,** Thibaut Léger **1,***

1 : ANSES Fougères, Unité de Toxicologie des Contaminants, 10B rue Claude Bourgelat, PA de la Grande Marche, Javené CS 40608 35306 Fougères Cedex - France

* : Auteur correspondant

Climate change induces multiple effects on marine ecosystems, by changing the temperature, acidity and oxygen content of oceans. These ecological perturbations increased the frequency and range of harmful algal blooms, linked to public health concern. Among the harmful dinoflagellates, *Vulcanodinium rugosum*, recently discovered in France, can produce cyclic imine toxins, pinnatoxins and portimine, that are currently non-regulated. If accumulation of pinnatoxin-G was found in large amounts in mussels, portimine was not detected in high concentrations. Pinnatoxin-G is a neurotoxin inhibiting nicotinic acetylcholine receptors (nAChR) that induces a quick death in mice after intraperitoneal or oral administration. Pinnatoxin-G has been recently described to cross the intestinal barrier and to distribute inside the organism, reaching the brain, the spleen and the liver after oral treatment of rats. In contrast, portimine is a weak toxic agent in acute in vivo toxicity tests but has been shown to induce apoptosis in vitro in several cell lines. Recent results, in our laboratory, indicated that apoptosis was not induced by portimine in the liver cell line HepaRG but could possibly induce a DNA replication stress and/or be detoxified by HepaRG cells. Studying the effects of these two toxins on liver is particularly relevant because it is the main organ implicated in xenobiotic metabolism. We used an integrative multi-omics methodology following a 24h treatment of HepaRG cells: proteomics allows characterizing toxin effects and the pathways involved and metabolomics brings new insights into their potential metabolization.

Mots-Clés : Multiomics ; Proteomics ; Metabolomics ; Portimine ; PinnatoxinG ; HepaRG ; Liver

Glass fiber membranes as stationary phase for lateral flow assays. Immobilization of complex lipoprotein vesicles in the test-line for cyclic imine detection.

Romulo Araoz **1,2,** Michaël Trichet **3,** Denis Servent **1, 2**

1 : SIMoS/ Laboratoire Toxines, récepteurs et canaux ioniques, CEA-Saclay, SIMoS, Bâtiment 152, 91191 Gif-sur-Yvette, France
2 : CNRS, ERL9004, CNRS DR4
3 : Sorbonne Université, CNRS, SME, IBPS, F-75005 Paris

NeuroTorp lateral flow assay is a functional method based on the mechanism of action of competitive antagonism of cyclic imine toxins towards muscle-type nicotinic acetylcholine receptors. When applied to the sample pad, cyclic imine toxins will compete with the toxin-tracer for the acetylcholine-binding site of nicotinic receptors. The degree of inhibition is concentration dependent. Innovative aspects of NeuroTorp are the use of glass microfiber membranes as stationary phase for lateral flow assays and the immobilization of Torpedo marmorata electrocyte membranes as a source of nicotinic acetylcholine receptors in the test-line. GF/C glass microfiber filter is an inert porous membrane with low unspecific binding currently used for preparing the conjugate pad that uniformly release the detector reagent into the mobile phase in lateral flow assays. Torpedo-electrocyte membranes is a validated source of nicotinic receptors of muscle-type. The sacrifice of an electric fish provide enough material to produce hundred thousand NeuroTorp test strips. Scanning electron microscopy studies enabled the elucidation of the mechanism by which Torpedo-electrocyte membranes are immobilized. Following application, the Torpedo-electrocyte membrane vesicles are retained in the voids of GF/C microfibers and after collapsing, the membranes anchor onto the neighboring microfibers randomly placed at the same plane forming lamellar membrane structures. The resulting novel nanocomposite interphase resists drying and is stable for years providing an optimal environment for the interaction of nicotinic ligands with their receptor target. Scanning electron microscopy also corroborated the specific interaction of α-bungarotoxin with the anchored Torpedo-nicotinic acetylcholine receptor. Taking all together, NeuroTorp biotechnology paves the way for immobilizing multiprotein complexes and cellular membranes on the test-line of lateral flow assays rather than a single biological or chemical macromolecule.

Acknowledgements: We thank the NRBC-E Program (MULTITOX project), the INTERREG Atlantic Area (ALERTOX-NET project), the LABEX LERMIT (DETECTNEUROTOX project) and the GDR PHYCOTOX.

Mots-Clés : Torpedo ; electrocyte membranes ; lateral flow assay ; cyclic imine toxins ; ligand binding assay ; scanning electron microscopy

First subcellular localization of the amnesic shellfish toxin, domoic acid, in bivalve tissues: deciphering the physiological mechanisms involved in its long-retention in the king scallop *Pecten maximus*

José Corona **1**

1 : Laboratoire des Sciences de l'Environnement Marin (LEMAR-UMR6539), Institut Universitaire Européen de la Mer, Université de Bretagne Occidentale, LEMAR, UMR 6539 UBO, Technopôle Brest-Iroise, 29280 Plouzané, France. - France

Domoic acid (DA), the phycotoxin responsible for amnesic shellfish poisoning (ASP), is an excitatory amino acid naturally produced by at least twenty-eight species of the bloom-forming marine diatoms *Pseudo-nitzschia* spp. Suspension feeders, such as bivalve mollusks, can accumulate and lengthy retain high amounts of DA in their tissues, threatening human health and leading to extensive-prolonged fishery closures, and severe economic losses. This is particularly problematic for the king scallop *Pecten maximus*, which retains high burdens of DA from months to years compared to other fast-depurator bivalves. Nonetheless, the physiological and cellular processes responsible for this retention are still unknown. In this work, for the first time, a novel immunohistochemical techniques based on the use of an anti-DA antibody was successfully developed and applied for DA-detection in bivalve tissues at a subcellular level. Our results show that in naturally contaminated *P. maximus* following a *Pseudo-nitzschia australis* outbreak, DA is visualized mainly within small membrane-bounded vesicles (1 – 2.5 µm) within the digestive gland cells, identified as autophagosomic structures by means of immune-electron microscopy, as well as in the mucus-producing cells, particularly those from gonad ducts and digestive tract. Trapping of DA in autophagososomes may be a key mechanism in the long retention of DA in scallops. These results and the development of DA-immunodetection are essential to provide a better understanding of the fate of DA, and further characterize DA contamination-decontamination kinetics in marine bivalves, as well as the main mechanisms involved in the long retention of this toxin in P. maximus.

Mots-Clés : Amnesic Shellfish Poisoning ; domoic acid ; immunodetection ; toxicokinetics ; scallops ; autophagosomes.

Transfer Learning for Marine Ecosystem Monitoring: focus on the automated recognition of HABs in the English Channel with the 'EcoTransLearn' R-package

Guillaume Wacquet **1 ***, Alain Lefebvre **1**

1 : IFREMER - Laboratoire Environnement Ressources, 150 Quai Gambetta, 62200 Boulogne-sur-Mer - France

* : Auteur correspondant

In recent years, Deep Learning (DL) has been growing in popularity in many fields, and in particular in image recognition, thanks to its capability to solve problems that have resisted the best attempts of the « classical » machine learning methodologies for many years. However, building an appropriate DL model from scratch in the frame of marine ecosystem monitoring, especially for plankton recognition, represents a challenging task, due to the dynamic nature and the morphological variability of the living particles, but also to the expensive cost in terms of time, human resources and skills to label a large number of training images. To overcome this problem, Transfer Learning (TL) can be used to improve a classifier from one domain with small amount of training data, by transferring information learned from a different domain on a large amount of training data. For this purpose, the 'EcoTransLearn' R-package is being developed to allow greater automation in classification of images acquired with various devices, thanks to different TL methods pre-trained on the generic ImageNet dataset. As a proof-of-concept, this R-package was used to analyse datasets acquired with a FlowCam system during cruises and within monitoring networks. The experimental results can then be used to evaluate the operational ability to automatically monitor the diversity of samples for the micro-phytoplankton in near-real time during cruises, and also to detect, track and count the most frequent potentially harmful algae found in the English Channel, like species belonging to the genera *Pseudo-nitzschia* and *Phaeocystis* in the frame of monitoring networks. Due to its simplicity and speed of analysis, this tool could be an integral part of a more general decision support system, by being coupled with other machine learning tools using integrated environmental data (from buoys, satellite, modelling) for an earlier prediction and tracking of harmful algal blooms.

Mots-Clés : Transfer Learning ; Image Recognition ; Automated Classification ; HAB ; English Channel

La gouvernance pour lutter contre les impacts des efflorescences algales nuisibles. Le cas de la pêcherie française à la coquille Saint-Jacques en Manche-est

Jose Perez **1 *,** Sarra Chenouf, Pascal Raux

1 : AMURE, Ifremer, Brest, Université de Brest Occidentale (UBO), CNRS, Technopôle Brest-Iroise, Rue Dumont d'Urville, 29280 Plouzané - France

* : Auteur correspondant

Les efflorescences d'algues nuisibles (HABs) sont des phénomènes qui peuvent avoir des impacts sur la biodiversité et sur les usages anthropiques qui ont lieu dans les zones maritimes et côtières. Les activités primaires, telles que la pêche aux coquillages, sont fortement concernées par ces événements en raison des interdictions de production et de commercialisation visant à éviter des intoxications alimentaires. Pour faire face à ce problème, la gouvernance des HABs repose sur deux piliers. Le premier s'appuie sur un système de surveillance de la qualité de l'eau afin d'évaluer les risques de contamination des eaux côtières par les HAB. Le second consiste en un système réglementaire de fermetures administratives des zones impactées. Cette action publique a deux objectifs. Le premier objectif est d'ordre sanitaire et vise à préserver la santé humaine. Le second est d'ordre économique et vise à minimiser les impacts économiques liés aux interdictions de commercialisation subies par les entreprises concernées. Ces objectifs sont a priori antagoniques. En s'appuyant sur le cas d'étude de cas de la pêcherie française de coquilles Saint-Jacques en Manche orientale et en se basant sur une analyse des risques de HAB et des impacts économiques potentiels associés, cette présentation analyse la gouvernance des HAB en France pour questionner ses forces et faiblesses. Cette gouvernance ne se résume pas à l'application mécanistique de fermetures administratives liées au dépassement de seuils de toxicité, mais il s'agit d'un processus de décision dynamique entre experts scientifiques et l'Administration dans la perspective de chercher un équilibre entre l'acceptabilité des risques sanitaires et des impacts économiques.

Mots-Clés : HABs ; Gouvernance ; coquille Saint ; Jacques ; pêcherie

BiRHaM project - Effects of harmful microalgae on reproduction of commercial bivalves.

Fernando Ramos Queiroga **1*,** Caroline Fabioux **1*,** Nelly Le Goic **1,** Malwenn Lassudrie **2,** Hélène Hégaret **1**

1 : Laboratoire des Sciences de l'Environnement Marin (LEMAR), Institut Universitaire Européen de la Mer : UMR6539, Technopôle Brest-Iroise - Rue Dumont d'Úrville - 29280. Plouzané - France
2 : Institut Français de Recherche pour l'Exploitation de la Mer, Station de Biologie Marine - BP 225 Place de la Croix - 29182 Concarneau - France

* : Auteur correspondant

Bivalves are key component of coastal ecosystems and constitute an important resource for human food. The progress of this mollusks farming is extremely dependent of our understanding of its physiological characters and its responses to environmental variables. The bivalve culture represents a profitable and eco-friendly activity especially because it does not require the addition of food, as they filter feed on the local plankton, including the microalgae and harmful algae. Unfortunately, some observed losses in the bivalve stock renewal can be hypothetically associated to occurrence of harmful algal blooms (HABs) because the gonadal maturity and spawning season of most commercial bivalve species coincided with the occurrence of HABs. Thus, bivalves can filter these harmful microalgae and retain toxins that can interfere with animal physiology and gametogenesis. Gametes and embryos, once released in the water column can be directly exposed to these microalgae and their toxins, thus affecting fertilization and development. The aim of the BiRHaM project is to characterize the effect of some toxic microalgae on reproduction of main commercial bivalve species of Brittany. The first step consists in the in vitro exposition of gametes and embryos of different bivalve species to different algal cultures and strains to assess toxicity and changes in some physiological parameters: ROS production, mitochondrial membrane potential, activity of ABC transporters, percentage of fertilization and embryo-larval development. Bivalve/algal species couples will be selected for assays in the second stage, where bivalves will be fed with harmful microalgae cultures and compared with non-harmful microalgae. Again, gametes and embryos will be collected for analysis of the parameters mentioned above, as well as others (amounts of ATP, total content of proteins, carbohydrates, lipids, RNA, histopathology with light and electron microscopy). This study aims at identifying the microalgal species and toxins which can affect bivalve reproduction and recruitment in Brittany.

Mots-Clés : Gametes ; Fecundation ; Oxidative stress ; ABC transporters activity ; Gametogenesis.

Investigating leads to understand the long contamination of King Scallop (*Pecten maximus*) and the importance of considering bottom water in monitoring.

Margot Deléglise **1*,** Leynaert Aude **1,** Gaspard Delebecq **1,** Justine Castrec **1,** Marc Long **1,** Malwenn Lassudrie **2,** Amélie Derrien **2,** Aouregan Terre-Terillon **2,** Erwan Amice **1,** Thierry Le Bec **1,** Laurent Chauvaud **1,** Caroline Fabioux **1,** Hélène Hégaret **1**

1 : Laboratoire des Sciences de l'Environnement Marin (LEMAR), Institut de Recherche pour le Développement, Institut français de Recherche pour l'Exploitation de la Mer, Université de Brest : UMR6539, Centre National de la Recherche Scientifique, Technopôle Brest-Iroise - rue Dumont d'Urville - 29280 Plouzané - FRANCE - France

2 : Ifremer, LER-BO, Station de Biologie Marine – Place de la Croix – BP 40537 – 29185 CONCARNEAU Cedex - France

* : Auteur correspondant

With an increase in harmful algae algal bloom episodes over the years, shellfish farming and fishermen are facing closures of ring fishing seasons. These are always more numerous and longer. In France, this industry was is subjected to an increase of blooms of Pseudo-nitzschia and its toxin domoic acid (DA). The exploitation of the king scallop (*Pecten maximus*) is one of the most affected fisheries. Unlike most bivalves, *P. maximus* takes very long time to depurate DA, up to years in some cases.

Since 2011, a bi-monthly in situ survey has been deployed in the Bay of Brest (France) to assess for the presence of *Pseudo-nitzschia* cells and DA in surface and bottom waters, as well as a measurement of DA in king scallops. The objective of this monitoring is to informations collected could be a lead to favor the understanding of the long DA contamination of scallops, which could be partly explained by the presence at the bottom of the water column of *Pseudo-nitzschia* cells, or dissolved DA, not detectable by classical sub-surface water samplings.

Preliminary results demonstrate that *Pseudo-nitzschia* cell and DA can sometimes be detected in the bottom water and as well as also in shellfish in scallops, even though there is no sign of *Pseudo-nitzschia* or DA at the surface. Furthermore, DA contamination in scallops contamination seems to be dependent on DA concentration from both surface and bottom waters. These results highlight the importance to consider both surface and bottom water sampling, when developing monitoring strategies of benthic bivalve species.

Mots-Clés : Harmful algae algal bloom ; HAB ; domoic acid ; monitoring ; *Pecten maximus* ; *Pseudo nitzschia*.

Interactions between filter-feeding bivalves and toxic diatoms: influence on the feeding behavior of *Crassostrea gigas* and *Pecten maximus* and on toxin production by *Pseudo-nitzschia*

Aurore Sauvey **1*,** Françoise Denis **2,** Hélène Hégaret **3,** Bertrand Le Roy **1,** Christophe Lelong **4,** Orianne Jolly **5,** Marie Pavie **1,** Juliette Fauchot **1**

1 : UMR BOREA - Biologie des Organismes et Ecosystèmes Aquatiques, Normandie Univ, UNICAEN, MNHN, Sorbonne Université Paris 6, Université des Antilles-Guyane, CNRS : UMR8067, Institut de recherche pour le développement [IRD] : UR207, Esplanade de la Paix – CS 14032 14032 CAEN Cedex 5 - France

2 : Biologie des Organismes et Ecosystèmes Aquatiques, Muséum National d'Histoire Naturelle (MNHN), CNRS : UMR8067, Sorbonne Université UPMC Paris VI, Institut de recherche pour le développement [IRD] : UR207, Université de Caen Normandie, Université des Antilles-Guyane, Station de biologie marine, MNHN, 29900 Concarneau - France

3 : Laboratoire des Sciences de l'Environnement Marin (LEMAR), Institut de Recherche pour le Développement, Institut français de Recherche pour l'Exploitation de la Mer, Université de Brest : UMR6539, Centre National de la Recherche Scientifique, Université de Brest, Centre National de la Recherche Scientifique : UMR6539, IUEM Technopôle Brest-Iroise - rue Dumont d'Urville - 29280 Plouzané - FRANCE - France

4 : Œstrogènes, reproduction, cancer, Université de Caen Normandie : EA2608 - UFR des Sciences - Esplanade de la Paix - CS 1403214032 Caen cedex 5 - France

5 : Crec - Station marine, Université de Caen-Normandie, Station marine, 54 Rue du Docteur Charcot 14530 Luc sur mer, CS 14032, 14032 Caen cedex 05 - France

* : Auteur correspondant

Among *Pseudo-nitzschia* species, some produce the neurotoxin domoic acid (DA), a source of serious health problems for marine organisms. Filter-feeding organisms – e.g. bivalves feeding on toxigenic *Pseudo-nitzschia* spp. – are the main vector of DA in humans. However, little is known about the interactions between bivalve and *Pseudo-nitzschia*. In this study, we examined the interactions between two juvenile bivalve species – oyster (*Crassostrea gigas*) and scallop (*Pecten maximus*) – and two toxic *Pseudo-nitzschia* species – *P. australis* and *P. fraudulenta*. We characterized the influence of (1) diet composition and the *Pseudo-nitzschia* DA content on the feeding rates of oysters and scallops, and (2) the presence of bivalves on *Pseudo-nitzschia* toxin production. Both bivalve species fed on *P. australis* and *P. fraudulenta*. However, they preferentially filtered the non-toxic *Tisochrysis galbana* compared to *Pseudo-nitzschia*. The presence of the most toxic *P. australis* species resulted in a decreased clearance rate in *C. gigas*. The two bivalve species accumulated DA in their tissues (up to 0.35 × 10-3 and 5.1 × 10-3 µg g-1 for *C. gigas* and *P. maximus*, respectively). Most importantly, the presence of bivalves induced an increase in the cellular DA contents of both *Pseudo-nitzschia* species (up to 58-fold in *P. fraudulenta* in the presence of *C. gigas*). The results of this study highlight complex interactions that can influence toxin production by *Pseudo-nitzschia* and accumulation in bivalves. These results will help to better understand the biotic factors that drive DA production by *Pseudo-nitzschia* and bivalve contamination during *Pseudo-nitzschia* blooms.

Mots-Clés : domoic acid ; filter ; feeding bivalves ; *Pseudo nitzschia* ; interactions ; filtration ; toxin accumulation ; *Crassostrea gigas* ; *Pecten maximus*

Gambierdiscus excentricus, a ciguatoxin producer? (Results of the project ExcenTox)

Thomas Yon **1,** Korian Lhaute **1,** Jérôme Viallon **2,** Rachelle Lanceleur **3,** Sarah Cambert **3,** Kevin Hogeveen **3,** Taiana Darius **2,** Valérie Fessard **3,** Damien Reveillon **1,** Manoella Sibat-Dubois **1,** Mireille Chinain **2,** Samuel Bertrand **4,** Philipp Hess **5**

1 : Laboratoire métabolites des microalgues (METALG), IFREMER, Centre de Nantes, Rue de l'île d'yeu 44311 Nantes cedex 03 - France
2 : Institut Louis Malardé - UMR 241 EIO, Laboratoire des Biotoxines Marines, PO Box 30, 98713 Papeete, Tahiti - Polynésie française
3 : Laboratoire de Fougères – ANSES, 10B rue Claude Bourgelat, Javené CS 40608 35306 Fougères Cedex - France
4 : Institut Des Substances et Organismes de la Mer, Nantes Université - UFR des Sciences Pharmaceutiques et Biologiques, 2, rue de la Houssinière BP 81227 44322 NANTES CEDEX 3 - France
5 : Physiologie et Toxines des Microalgues Toxiques et Nuisibles, IFREMER, Centre de Nantes, Rue de l'Île d'Yeu 44311 Nantes - France

---

Toxic benthic algae, especially those belonging to the genus *Gambierdiscus*, produce various toxins (ciguatoxins (CTXs), maitotoxins (MTXs), gambierones, gambieric acids...). CTXs can accumulate in fish and seafood and cause intoxication known as ciguatera poisoning (CP). The high diversity and discovery of new species of *Gambierdiscus* in the Atlantic Ocean (Canary Islands, French West Indies, East Coast of the United States) demonstrate the need to identify the risks associated with these microalgae potentially involved in CP. Since 2011, the evaluation of the toxicity of different *Gambierdiscus* species in the Atlantic Ocean has been carried out mainly with bioassays such as the mouse neuroblastoma cell-based assay (CBA-N2a) and the results suggested the species *Gambierdiscus excentricus* and *Gambierdiscus australes* as potential CTX producers.
The ExcenTox project aimed to identify the toxin production of the strain of *G. excentricus* Pulley-Ridge Gam 2 whose total activity was estimated to be 469 fg CTX3C eqv/cell by Litaker et al. (2017). First, two chemical extraction protocols based on liquid/liquid partition and fractionation were used. Second, *G. excentricus* extracts and purified fractions were tested in three independent experiments using three different CBA-N2a protocols in three laboratories, the Phycotoxins laboratory (Ifremer), the Fougères laboratory (Anses), and the Marine Biotoxins laboratory (ILM). Results were compared and statistically analysed.
Sensitivity and reproducibility of each CBA-N2a protocol were analysed showing better results for pure toxins tested (CTX3C, MTX1, purified MTX4) compared to *G. excentricus* extracts and fractions. Even if significant differences were observed for most EC50 values of pure toxins and *G. excentricus* extracts obtained with the three CBA-N2a protocols, this project demonstrated a good concordance of the results between the three laboratories highlighting the absence of CTX-like cytotoxicity in G. excentricus extracts and fractions and a cytotoxicity of the purified MTX4 at least 7 times higher than that of MTX1 standard.

Mots-Clés : ciguatera ; *Gambierdiscus excentricus* ; bioassay ; bioguided fractionation ; maitotoxin ; ciguatoxin

Gambierol effects on k+ currents and catecholamine release in single rat fetal adrenomedullary cultured chromaffin cells

Evelyne Benoit **1,** Sébastien Schlumberger **2,** Jordi Molgó **1*,** Makoto Sasaki **3,** Haruhiko Fuwa **4,** Roland Bournaud **2**

1 : Service d'Ingénierie Moléculaire pour la Santé (SIMoS), Université Paris-Saclay, CEA, Département Médicaments et Technologies pour la Santé (DMTS), Institut des Sciences du Vivant Frédéric Joliot, ERL CNRS 9004 , F-91191 Gif-sur-Yvette - France
2 : Laboratoire de neurobiologie cellulaire et moléculaire, CNRS UPR9040, F-91198 Gif-sur-Yvette - France
3 : Graduate School of Life Sciences, Tohoku University, 980-8577 Sendai - Japan
4 : Department of Applied Chemistry, Faculty of Science and Engineering, Chuo University, 112-8551 Tokyo - Japan

* : Auteur correspondant

The purpose of this work was to study the action of the polycyclic ether gambierol on K+ currents and catecholamine secretion in single rat fetal adrenomedullary cultured chromaffin (AMC) cells using perforated whole-cell voltage-clamp recordings, and current-clamp and electrochemical recordings. From the several types of voltage-gated K+ channels (KV) contributing to the total outward current of rat fetal AMC cells, gambierol only partly inhibited the total K+ current, when added after or before KCa and KATP blockers, and affected neither KCa nor KATP channels. After blocking of Nav and KATP channels, and preventing the activation of KCa channels, gambierol blocked 50% of the maximal K+ current fraction with an inhibitory concentration (IC50) of 5.8 ± 1.56 nM (n = 9). In marked contrast to ciguatoxins, gambierol slowed the kinetics of K+-current activation by 75.4 ± 10.1% (n = 4) with respect to controls (p = 0.031). Hence, before and after gambierol the activation time constants of K+ current were 3.82 ± 0.39 ms (n = 4) and 6.80 ± 1.02 ms (n = 4), respectively. Simultaneous current-clamp and single-cell amperometry recordings revealed that gambierol did not modify the membrane potential following 11-seconds depolarizing current-steps, in both quiescent and active cells displaying repetitive firing of action potentials, and it did not increase the number of exocytotic catecholamine release events, with respect to controls. The subsequent block of KCa channels, both depolarized the membrane and enhanced by 2.7 and 3.5-fold the exocytotic event frequency in quiescent and active cells respectively, highlighting the key modulatory role played by KCa channels in the control of exocytosis from rat fetal AMC cells

---

Mots-Clés : gambierol ; potassium currents ; calcium ; activated K+ channels ; ATP ; sensitive K+ channels ; catecholamine release

Determination of toxicity and toxins diversity of harmful benthic species associated with ciguatera collected in the French West Indies (Caribbean Sea)

Manoella Sibat **1,** Korian Lhaute **1,** Gwenaël Bilien **2,** Nicolas Chomérat **2,** Aurélie Boisnoir **3**

1 : Laboratoire métabolites des microalgues (METALG), IFREMER, Centre de Nantes rue de l'île d'yeu 44311 Nantes cedex 03 - France

2 : Laboratoire Environnement Ressources de Bretagne Occidentale (LER-BO), IFREMER, Station de biologie marine Place de la Croix 29185 Concarneau cedex - France

3 : Unité Biodiversité et Environnement (BIODIVENV), Station Ifremer Martinique 79 route de Pointe Fort 97231 Le Robert - Martinique

---

Ciguatera poisoning caused by the consumption of seafood contaminated with ciguatoxins (CTX), is a major concern and on the rise in the French West Indies. These neurotoxins are produced by the genera *Gambierdiscus* and *Fukuyoa*, but other toxic epiphytic dinoflagellates are generally associated with ciguatera, such as *Ostreopsis* spp. or *Coolia* spp. A study was conducted on these organisms in the French West Indies to characterize their diversity, toxicity and toxin profile in order to better understand and manage the risk for populations and economic activities. Epiphytic cells were collected in Saint Barthelemy, Saint Martin, Guadeloupe and Martinique islands, and isolated to establish monospecific cultures. Taxonomic identifications were undertaken by using a morpho-molecular approach. Toxicity of the strains was assessed with neuroblasma cell based assays (N2a) and toxin profiles were characterized by liquid chromatography coupled to tandem mass spectrometry (LC-MS/MS). The study allowed to identify 3 species of *Gambierdiscus* (*G. silvae, G. belizeanus* and *G. caribaeus*) and *Fukuyoa ruetzleri*, 3 species of *Coolia* (*C. tropicalis, C. santacroce* and *C. malayensis*), and 2 species of *Ostreopsis* (*O. siamensis* and *O.* cf. *ovata*) by molecular analysis. CTX-like activity was evaluated on the *Gambierdiscus*, *Coolia* and *Fukuyoa* strains only *G. silvae* exhibited a toxicity but no known CTX were found. LC-MS/MS analyses were carried out on 27 strains and major results were highlighted: (i) determination of three distinct toxin profiles for *Gambierdiscus* species, (ii) two putative new isomers of 44-methyl gambierone were detected in a strain of *C. tropicalis*, (iii) ovatoxin-a, -b and -e were present in one strain of *O.* cf. *ovata* and (iv) ostreocin-A, -D, and a new putative ostreocin were detected in O. siamensis. These results unveiled the biodiversity and toxins diversity of the species associated with Ciguatera in the French West Indies.

Mots-Clés : Ciguatera ; *Gambierdiscus* ; *Ostreopsis* ; *Coolia* ; LCMS/MS ; toxins diversity ; N2A

Characterization of phytoplankton cells using dielectrophoresis – a promising method for the study of harmful algal blooms

Agathe Dujardin **1,** Lylian Challier **1*,** Cecile Jauzein **2,** Vincent Noel **3**

1 : Laboratoire Détection Capteurs et Mesures, IFREMER, 1625 Rte de Sainte-Anne, 29280 Plouzané - France
2 : Laboratoire Dyneco Pelagos, IFREMER, 1625 Rte de Sainte-Anne, 29280 Plouzané - France
3 : Laboratoire Itodys, Université de Paris, 5 Rue Jean Antoine de Baïf, 75013 Paris - France

* : Auteur correspondant

In aquatic systems, Harmful Algal Blooms (HABs) may represent a risk for public health and have deleterious impacts on economic activities (fisheries, tourism...). In France, management and mitigation of HABs mainly relies on monitoring networks that use microscopic observations for identification and counting of toxic phytoplankton species. If microscopy is an efficient tool, it is highly time consuming and requires strong expertise. Flow cytometry is another useful method for the monitoring of phytoplankton communities. Discriminating cells according to their size, fluorescence and global structure, it enables counting and characterization of main phytoplankton groups but does not allow for seperating co-occurring species that have close morphological characteristics. A new sorter system, able to sort microalgae at the species level without a need for chemical fixation or labelling, will create an undeniable advantage for the study and mitigation of HABs.
Dielectrophoresis is a promising tool in this context. This methodological approach used inhomogeneous oscillating electric fields in microfluidic channels: cells in suspension are either attracted to or repulsed by electrodes, according to their dielectric properties that are dependent on cellular size, structure and chemical composition. Associated analytical devices allow for isolation and sorting of particles in a high flow-rate and without using any kind of membrane.
Microprinted prototypes were tested for characterizing two species of dinoflagellates that have highly close morphologies: *Alexandrium minutum* (paralytic shellfish toxins producer) and *Scrippsiella acuminata* (non toxic). Dielectric profiles obtained were different for the two strains tested. The dielectrophoretic signature of microalgal cells was further analysed according to growth phases. These results are highly promising, depicting dielectrophoresis as a potential new mode of cell sorting. Major contributions of this method can be foreseen in the frame of both management of HABs (early detection in particular) and/or ecophysiological characterization of microalgal cells (cell response to environmental stressors).

Mots-Clés : microalgae ; dielectrophoresis ; cell sorting ; microfluidics

Environmental factors driving *Pseudo-nitzschia* sp. blooms in the Seine estuary

Angéline Lefran **1*,** Pascal Claquin **2,3,** Tania Hernandez Fariñas **1**

1 : IFREMER Laboratoire Environnement Ressources de Normandie, LITTORAL, Avenue du Général de Gaulle 14520 Port en Bessin - France

2 : Biologie des ORganismes et Ecosystèmes Aquatiques (BOREA), Université de Caen Basse-Normandie, Muséum National d'Histoire Naturelle (MNHN), CNRS : UMR7208, Institut de Biologie Fondamentale et Appliquée Université de Caen Basse-Normandie, Esplanade de la Paix CS 14032 14032 CAEN cedex 5 - France

3 : Centre de recherches en environnement côtier, Université de Caen Normandie, 54, rue du Docteur Charcot - 14530 Luc-sur-Mer - BP 49 - France

* : Auteur correspondant

Harmful Algal Blooms (HABs) are frequent events affecting shellfish's production and harvest, causing ecological and economic issues. Among them, *Pseudo-nitzschia* sp. is a chain forming diatom at the origin of Amnesic Shellfish Poisoning (ASP) due to domoic acid (DA) production. Studies have shown that nutrient inputs, temperature and salinity are major drivers of this genus, which can bloom in both spring and autumn. Through both experimental and historical approaches, this work aimed at understanding the bloom dynamic and its toxicity potential, considering its surrounding environment at the Seine estuary, in spring 2019, and over the period 2005-2019. For the first approach, a two-steps campaign (pre- and post-bloom) aimed at exploring the effects of the Seine river's plume onto a spring bloom distribution and toxicity in 2019. For the second, the database related to the REPHY monitoring program was used in order to access high-resolution time-series of *Pseudo-nitzschia* sp. abundance, its toxicity and related environmental parameters at two stations near the Seine estuary. Through this last approach, the study aimed at comparing the environmental conditions surrounding the annual blooms over fifteen years through ecological niche analysis. Indeed, while *Pseudo-nitzschia* sp. is a cosmopolite and regular blooming species, conditions for DA toxicity in shellfish were only found between 2011 and 2014. Our first results show that in 2019 *Pseudo-nitzschia* sp.'s had a coherent location within the most productive zone of the Seine river's mouth, at the interface between irradiance limitation (river's plume) and nutrient limitation (outer bay area). The historical approach highlighted that trajectories behind species' dynamics are not straight; niches are flexible within a changing environment. The ecological niche analysis on *Pseudo-nitzschia* sp. suggests that specifically during the period known for DA toxicity, the genus was constrained by biotic interactions, which was not the case during neither periods before nor after.

Mots-Clés : *Pseudo nitzschia* ; ecological niche ; environmental drivers ; toxicity

Influence of phosphorus conditions on the growth and phagotrophy of the ichthyotoxic haptophyte *Prymnesium parvum*

Clémence Boucher **1,** Julie André **1*,** Damien Reveillon **1*,** Thomas Lacour **1***, Per Juel Hansen **2*,** Francis Mairet **1***

1 : IFREMER Centre atlantique, Rue de l'Île d'Yeu 44311 Nantes Cedex 03 - France
2 : University of Copenhagen, Department of Biology, Marine Biological Section, Strandpromenaden 5 3000 Helsingør - Danemark

* : Auteur correspondant

The haptophyte *Prymnesium parvum* can form Harmful Algal Blooms (HABs) and produce toxic compounds that lead to fish kills, affecting the food web. This ichtyotoxic microalgae is globally distributed and thus of significant ecological importance. The main nutritional mechanism of P. parvum is mixotrophy, which combines photoautrophy and phagotrophy. This strategy enables some species to be able to persist by feeding on organic particles. To further investigate the effects of changing nutritive conditions on the growth and physiological state of *P. parvum*, laboratory experiments were conducted over a month on a phosphorus-deficient *P. parvum* culture grown with or without addition of phosphorus (P). Two sources of P were compared: inorganic nutrient and/or algal prey (i.e. the cryptophyte *Teleaulax amphioxeia*). Detection and quantification of the phagotrophic activity of *P. parvum* was performed using a combination of confocal microscopic observations, flow cytometry fluorescence measurements and cell sorting. The feeding rate of *P. parvum* on *T. amphioxeia* was calculated based on the phycoerythrin (PE) fluorescence signal. The mean growth rate of *P. parvum* was higher with the addition of prey than with inorganic P (0.58 vs. 0.42 d-1) and was maximal when both sources of P were added (0.79 d-1). The amount of *T. amphioxeia* ingested per *P. parvum* per day (Ta Pp-1 d-1) reached a maximum of 0.4 and 0.66 when grown with and without addition of inorganic P, respectively. Furthermore, our results suggest that the ingestion rate depends on the prey/predator ratio, rather than on the abundance of prey. Further experiments assessing the production of toxic compounds will allow us to study the link between mixotrophy, toxicity, growth and the physiological state of P. parvum.

Mots-Clés : Mixotrophy ; Phagotrophy ; Phosphorus ; *Prymnesium parvum* ; Toxic

Bloom initiation dynamics of the green dinoflagellate *Lepidodinium chlorophorum* assessed by environmental DNA

Pauline Roux **1,** Mathilde Schapira **1,** Kenneth Neil Mertens **2,** Coralie André **2,** Aouregan Terre-Terillon **2**, Anne Schmitt **1,** Soazig Manach **1**, Karine Collin **1,** Joelle Serghine **3,** Cyril Noel **4,** Raffaele Siano **3***

1 : IFREMER, Laboratoire Environnement Ressources Morbihan Pays de Loire (LER-MPL), Centre Atlantique, rue de l'Ile d'Yeu, 44311 Nantes Cédex 3 - France
2 : Ifremer, LER-BO, Station de Biologie Marine – Place de la Croix – BP 40537 – 29185 CONCARNEAU Cedex - France
3 : Ifremer, Dyneco-Pelagos, Ifremer, Plouzané - France
4 : IFREMER, IRSI, Service de Bioinformatique (SeBiMER), Plouzané - France

* : Auteur correspondant

Green seawater discolorations caused by the marine dinoflagellate *Lepidodinium chlorophorum* have been frequently observed along Southern Brittany (NE Atlantic, France). This study aims at a better understanding of the dynamics of the L. chlorophorum bloom initiation using the metabarcoding of the environmental DNA (eDNA). Sediments (eDNA and cyst samples) collected in January-February 2019 and water samples from two stations collected at three water depths in September-March 2019-2020 and 2020-2021 (eDNA and environmental parameters) were analysed. The protistan community was dominated by dinoflagellates and was homogenous in the water column. Amplicon Sequence variants (ASVs) associated to the genus *Lepidodinium* were detected in winter at low relative abundances (minimum: 0.01%). Increase in *Lepidodinium* abundances were positively correlated with pulses of ammonium re-suspended from bottom sediments. Although *Lepidodinium* eDNA (<1%) was detected in the sediments, no cyst morphotypes could be associated to *Lepidodinium*, nor germination experiments revealed *Lepidodinium*-like cells, questioning the existence of sexual resting cysts of this species in the seed bank. It is hypothesized that temporary *Lepidodinium* cells remained present in the water column at low concentration during the autumn-winter period, awaiting ammonium input from sediments to initiate growth and that blooms develop when water column stratification and river input provide favourable environmental conditions for biomass increases.

Mots-Clés : *Lepidodinium chlorophorum* ; Green Seawater discoloration ; HABs ; Cysts ; Sediment resuspension ; eDNA ; Ammonium

Pseudo-nitzschia australis gene expression dynamic during a bloom

Léa Prigent **1*,** Julien Queré **1,** Martin Plus **1,** Mickael Le Gac **1***

1 : Ifremer, DYNECO-PELAGOS, Technopole Brest-Iroise, BP 70, 29280, Plouzané - France

* : Auteur correspondant

Pseudo-nitzschia blooms and the resulting toxicities are a global problem. Within this genus, many species produce domoic acid but major toxicity events are due to a few species, among which P. australis. The understanding of the toxicity events can therefore only be achieved by analyzing bloom dynamics at the species level. Ecological studies have provided valuable insights about the link between environmental variables and species successions in situ. However, the in situ physiology of the toxin producing species remains poorly characterized. This may now be addressed by using metatranscriptomic to determine gene expression dynamics of specific species during bloom events.
During one month, in April 2017, a metatranscriptomic sampling was carried out during a *P. australis* bloom in the Bay of Brest (Brittany, France). These samples were analyzed to investigate *P. australis* gene expression dynamics in situ. Using a global approach, several main functional gene categories displayed a strong temporal pattern of differential gene expression. For instance, genes involved in photosynthesis or carbon metabolism were highly expressed at the beginning and to a lesser extend at the end of the bloom. Genes involved in protein synthesis which may be associated with cell proliferation are most expressed in the middle of the bloom when *P. australis* relative abundances are high. Using a candidate gene approach, the expression of genes associated to domoic acid production, but also involved in sexual reproduction and nutrient uptake were also investigated. Altogether, this study is the first investigating gene expression dynamics of a harmful *Pseudo-nitzschia* species during the entire time course of a bloom. It highlights key functions displaying dynamic expression in situ and offers the promise of direct comparisons between the study of physiological processes in vitro and during blooms.

Mots-Clés : Gene expression dynamic ; Metatranscriptomic ; *Pseudo nitzschia australis* bloom ; Bay of Brest

Effects of small-scale turbulence on *Pseudo-nitzschia* spp.

Emilie Houliez **1*,** François Schmitt **1,** Vasileios Bampouris **1,** Clotilde Lequiniou **1,** Muriel Crouvoisier **1,** Amandine Caruana **2,** Juliette Fauchot **3,** Aurore Sauvey **3,** Urania Christaki **1**

1 : Laboratoire d'Océanologie et de Géosciences (LOG) - UMR 8187, Université du Littoral Côte d'Opale, CNRS : UMR8187, Université de Lille - Sciences et Technologies, 28 avenue Foch - 62930 Wimereux - France

2 : Laboratoire Physalg, PHYCOTOX, IFREMER, F-44000 - France

3 : Laboratoire BOREA, UMR 8067 MNHN, Sorbonne Université, UCN, CNRS, IRD-207, UA Museum National d'Histoire Naturelle (MNHN), CNRS, Institut de Recherche et Développement (IRD), Sorbonne Université (SU), Université de Caen Normandie (UCN), Université des Antilles (UA), 43 rue Cuvier, CP 26 75231 Paris Cedex 05 - France

* : Auteur correspondant

Some species of the diatom *Pseudo-nitzschia* are known to produce domoic acid, a neurotoxin that represents a threat to human health when it accumulates in consumed shellfish tissues. Currently, the factors favoring their growth and toxin production are not completely resolved. Like any phytoplankton species in the ocean, *Pseudo-nitzschia* spp. are continually exposed to the fluid flow and straining forces generated by small-scale turbulence due to winds, tidal currents and breaking waves. It is known that turbulence may affect plankton ecology and according to the famous Margalef's mandala turbulence may be favorable to diatoms. Turbulence affects most of the parameters that influence the diatoms survival including: settling velocity and cell re-suspension, nutrients acquisition and encounter-based processes such as interactions with grazers and diatom-diatom encounter rate for reproduction and chain formation. We hypothesized that turbulence may also influence *Pseudo-nitzschia* spp. physiology and blooms. To test this hypothesis, we run laboratory experiments to study the physiology and morphology of two *Pseudo-nitzschia* species (*P. multiseries* and *P. fraudulenta*) exposed to 5 levels of turbulence generated by an AGITURB system (ranging from still to storm conditions with Reynolds numbers from 130 to 360 and dissipation rates from 0.03 to 10 cm-2 s-3). Turbulence impacted the *Pseudo-nitzschia* growth rate and chain formation. The growth rate and formation of small chains (2 -3 cells) versus the Reynolds numbers presented a dome shape with highest values for moderate turbulence levels (Reynolds numbers from 180 to 270). This directly highlights that turbulence impacts *Pseudo-nitzschia* physiology and indicates that a moderate level of turbulence seems to offer the optimal conditions for their development.

Mots-Clés : *Pseudo nitzschia* ; turbulence ; harmful algal blooms ; amnesic shellfish poisoning ; domoic acid

Diversity of bacterial communities associated with *Ostreopsis* cf. *ovata* bloom in NW Mediterranean (COMBAC project)

Enora Briand **1*,** Rodolphe Lemée **2,** Elise Robert **1,** Florent Malo **3,** Damien Reveillon **4,** Fabienne Herve **4,** Nicolas Chomérat **5,** Gwenael Bilien **5,** Cyril Noel **6**

1 : PHYTOX, laboratoire GENALG, IFREMER, F-44000 Nantes - France
2 : CNRS, Laboratoire d'Océanographie de Villefranche, Université Paris-Sorbonne - Paris IV, F 06230 Villefranche-sur-mer - France
3 : PHYTOX, laboratoire PHYSALG, IFREMER, F-44000 Nantes - France
4 : PHYTOX, laboratoire METALG, IFREMER, F-44000 Nantes - France
5 : Unité Littorale, Laboratoire Environnement Ressources - Bretagne Occidentale, IFREMER, F 29185 Concarneau - France
6 : IRSI, SEBIMER, IFREMER, F-29280 Plouzané - France

* : Auteur correspondant

Microalgal-bacteria interactions occur in the microenvironment surrounding algal cells known as the phycosphere. In this region, the transformation of algal-derived organic matter, the exchange of metabolites and infochemicals govern phytoplankton-bacteria relationships, which span mutualism to antagonism. In the last decade, the increasing occurrence of extensive *Ostreopsis* cf. *ovata* blooms has been reported in temperate coastal regions, including the Mediterranean. This benthic dinoflagellate has been involved in human health problem probably related to its ability to produce palytoxin-like compounds, namely ovatoxins. Whereas abiotic factors, such as temperature, are well known to contribute to these proliferations, the role of biotic interactions between microalgae and bacteria are still poorly understood. Deciphering the bacterial community (BC) associated with *Ostreopsis* will help to understand the mechanisms underlying *Ostreopsis*-bacteria interactions that are essential to predicting dynamics of harmful algal bloom. This study investigated the bacterial diversity associated with *Ostreopsis* cf. *ovata* during different phases of a bloom development (pre-, during- and post-bloom) in NW Mediterranean (Villefranche bay, France). The BC composition (comparing free-living and microalgae-attached fractions) were assessed by a 16S metabarcoding approach. Overall, the BC was dominated by three classes: Bacteroidia (35%), Alpha- (37%) and Gamma-proteobacteria (28%). The fraction and bloom stages were identified as structuring factors of the *Ostreopsis* cf. *ovata* microbiome. Together, these results highlight shifts in BC composition with possible ecological and functional significance on *Ostreopsis* cf. *ovata* bloom dynamic.

Mots-Clés : *Ostreopsis* ; microbiome ; metabarcoding

Posters

BIOS project: using metabolomics to highlight biomarkers of (non)-exposition of mussel and oyster to phycotoxins

Valentin Derrien **1*,** Yann Guitton **2,** Amélie Derrien **3,** Virginie Le Razavet **4,** Florence Mondeguer **1,** Damien Reveillon **5**

1 : Ifremer, PHYTOX, laboratoire METALG, F-44000 Nantes - France
2 : Oniris, INRAE, LABERCA, Oniris, École vétérinaire, agroalimentaire et de l'alimentation, 44300 Nantes - France
3 : Ifremer, LER-BO, IFREMER, Station de Biologie Marine – Place de la Croix – BP 40537 – 29185 CONCARNEAU Cedex - France
4 : Ifremer, Plateforme Mollusques Marins de Bouin, F-85230 Bouin - France
5 : Ifremer, PHYTOX, laboratoire METALG, F-44000 Nantes - France

* : Auteur correspondant

Some micro-algae species produce toxins that can cause food poisoning in humans through the consumption of contaminated shellfish. As a result, monitoring programmes have been implemented to protect the consumers, based on the quantification of these phycotoxins by targeted analyses using liquid chromatography coupled to tandem mass spectrometry (LC-MS/MS). The main drawback of this approach comes from the focusing on a limited number of known toxins, which requires a constant need to update and validate the analytical methods. Alternatively, we could try to consider the consequences of a contamination by phycotoxins on the global metabolite expression of bivalves to find biomarkers of exposition or non-exposition.
In this context, the interest of metabolomics (i.e. the analysis of "all" the metabolites as the ultimate response of biological systems to environmental changes) will be investigated within the BIOS project. Contaminated and healthy mussels and oysters from the EMERGTOX network (i.e. the monitoring of both regulated and emerging phycotoxins in shellfish along the French coasts) and from Ifremer's facility will be analyzed on two high resolution mass spectrometers (Q-TOF and Q-Orbitrap thanks to a collaboration with the LABERCA). After proper data processing and statistical analyses, we hope to highlight some suitable biomarkers that will be tentatively identified by dereplication strategies such as molecular networks.

Mots-Clés : Metabolomics ; phycotoxins ; bivalves ; biomarkers ; monitoring programme

Tetrodotoxins in French Bivalve Mollusks: compilation of results from the projects ALERTOX-Net and TTX-Source

Véronique Savar **1,** Damien Reveillon **1*,** Laura Biessy **2*,** Kenneth Mertens **3*,** Kirsty Smith **2*,** Philipp Hess **4***

1 : Ifremer, PHYTOX, laboratoire METALG, F-44000 Nantes - France
2 : Cawthron Institute, Private Bag 2, Nelson, 7010 - Nouvelle-Zélande
3 : Ifremer, Laboratoire Environnement Ressources - Bretagne Occidentale, Concarneau - France
4 : Ifremer, METALG Laboratory, Unité PHYTOX, Rue de l'Île d'Yeu, 44000 Nantes - France

* : Auteur correspondant

During the last years the monitoring of TTXs in bivalves of European countries has attracted attention. To increase the knowledge about the relation between bacteria (suggested as a possible TTX source) and shellfish in France, we first optimized an analytical method based on hydrophilic interaction liquid chromatography coupled to tandem mass spectrometry (HILIC-MS/MS) to detect TTX and 5 analogs. Best recoveries in bacteria and oysters (total flesh) were obtained using a single extraction with 500 µL of 1% acetic acid followed by ultra-filtration (3 kDa for shellfish vs. 0.2 µm for bacteria). Matrix-matched calibration curves were used to cope with matrix effects (i.e. 41% enhancement of TTX signal in oyster and 255% in bacteria). To detect TTXs at best sensitivity (i.e. LOQ between 5-12.5 µg TTX /kg oyster), the Zic-Hilic (Merck) column and 10 mM of ammonium formate in aqueous mobile phase were chosen.
The HILIC-MS/MS method was then used to screen marine bacteria and bivalves collected along the French coasts. Importantly, no TTX was detected in the 122 strains of bacteria (mostly from the genus Vibrio) isolated from shellfish or environmental matrices or during mortality events. Interestingly, for oysters and clams, TTX was detected in June-July for 34% of shellfish collected in 2018 (n=35) and in 11% in 2019 (n=63) always at low levels (below the EFSA recommended limit of 44 µg TTX equivalent /kg).
In addition to 2019, we confirmed in 2021 the presence of TTX in oysters sampled in Kersanton, Brittany during June – July (n=3: 74 to 47 µgTTX /kg). Digestive glands contained most of the TTX (i.e. 424 vs. 16 µg/kg in remaining flesh the 24 of June), in agreement with a previous study.
We therefore reported new TTX occurrences in French shellfish. Regarding the potential producers, metabarcoding analyses are ongoing within the TTX-source project.

Mots-Clés : HILIC ; MS/MS ; TTXs ; bivalves ; bacteria ; metabarcoding

Which toxins of Ostreopsis cf. ovata, *Vulcanodinium rugosum* and *Karenia brevis* are responsible for skin irritation?

Loïc Plessis **1,2,** Damien Reveillon **1,** Florence Cassin **2,** Véronique Séchet **3**, Philipp Hess **1**

1 : Ifremer, METALG Laboratory, Unité PHYTOX, Rue de l'Île d'Yeu, 44000 Nantes - France
2 : Research & Innovation Direction, Yves Rocher, 7 chemin de Bretagne, 92130 Issy-les-Moulineaux, - France
3 : Ifremer, PHYSALG Laboratory, Unité PHYTOX, Rue de l'Île d'Yeu, 44000 Nantes - France

---

Several dinoflagellate genera are widely studied because of the toxins they produce. In addition to indirect health effects through the consumption of contaminated shellfish, harmful algal blooms (HABs) can also directly threaten human health through contact. However, respiratory and dermal toxicity of HABs is still poorly studied compared to shellfish poisoning, despite an increasing number of cases being recently reported.
This study focuses on deleterious effects on the skin, which have been linked to the species *Vulcanodinium rugosum*, *Ostreopsis* cf. *ovata* and *Karenia brevis*. These species can produce pinnatoxins/portimines, ovatoxins and brevetoxins, respectively. However, it is not yet clear whether these known toxins induce negative effects on human skin or whether other metabolites are involved. To clarify this question, we intend to characterise cytotoxicity, inflammatory response and oxidative stress in skin cells after a direct exposure to pure toxins and extracts of these three dinoflagellates. Using human keratinocytes (HaCaT) as a model, different bioassays will focus on biomarkers of inflammation (e.g. interleukines) and oxidative stress (e.g. intracellular ROS activity and genetic biomarkers). Preliminary results indicate significant cytotoxicity of portimine and ovatoxin as well as several extracts of *V. rugosum* and O. cf. *ovata*.

Mots-Clés : Phycotoxins ; Keratinocytes ; Oxidative stress ; Inflammation

NEMESIS thesis

Marie Deschler **1,2**

1 : Stress Environnementaux et BIOsurveillance des milieux aquatiques, Institut National de l'Environnement Industriel et des Risques : UMR-I 02, Université de Reims Champagne-Ardenne : CNRS3417, Université Le Havre Normandie : UMR-I 02, SFR CONDORCET, Université de Reims Champagne-Ardenne UMR-I 02 INERIS-URCA-ULH SEBIOUnité Stress Environnementaux et BIOsurveillance des milieux aquatiquesUFR Sciences Exactes et Naturelles, Campus du Moulin de la Housse, BP 103951687 Reims cedex 2 - France

2 : Biologie des Organismes et Ecosystèmes Aquatiques, CNRS : UMR8067 / UMR7208, Université des Antilles, Museum National d'Histoire Naturelle, Institut de Recherche pour le Développement, Sorbonne Université : UM95, Université de Caen Normandie, 7, rue Cuvier - CP 32 - 75231 Paris Cedex 05 - France

---

Diatoms of the genus *Pseudo-nitzschia* are present in many marine ecosystems including the Bay of Seine (BoS). They produce domoic acid (DA), a neurotoxin and are responsible for harmful algal blooms with human health and socio-economic consequences. Very few is known about the impact of domoic acid in food webs, especially for mesozooplankton mostly represented by copepods. These primary consumers may operate as a vector for the toxin toward higher trophic levels. The NEMESIS thesis and the INCIDENCE project are exploring for the first time in the BoS the interactions between Pseudo-nitzschia species and copepods. The reciprocal influence of both partners is studied under controlled laboratory experiments, focusing on physiology and behaviour. The chemical communication involved in this interaction will also be explored. Finally, an in situ approach will allow to validate laboratory observations. In a first experiment, *Pseudo-nitzschia australis*, the highest DA producer in the BoS, was exposed directly to the copepod Temora longicornis. The presence of copepods resulted in a higher toxin cell content in this diatom. This study suggests that biotic factors can modulate the toxicity of Pseudo-nitzschia blooms in the BoS, as previously shown in artic environments. However, other preliminary results suggest that all *Pseudo-nitzschia* species from the BoS may not respond to the presence of copepods by increasing their toxin production. Future experiments will therefore focus on the response of other *Pseudo-nitzschia* species from the BoS and will also use the model species *Eurytemora affinisto* explore physiological and behavioural response of copepods to toxic diatoms.

Mots-Clés : *Pseudo nitzschia* ; Copepods ; toxin